pool to be determined. To answer this question would require an inducible system in which Rag1-Cre expression can be temporally controlled.

Just like the adult lymphoid-primed multipotent progenitor (LMPP) identified by the same group a few years ago (Adolfsson et al., 2005), this Lin−Kit+Flt3+IL7Rα+ embryonic immune-restricted progenitor has GM, B, and T cell potentials, but no megakaryocyte-erythroid (MkE) potential. These findings suggest that one of the earliest hematopoietic lineage decisions is a restriction process of the lympho-myeloid lineage and a segregation of this potential from the MkE lineage. Why this lineage separation occurs at this early stage is currently unclear, but may be explained by an earlier requirement for large numbers of erythroid cells, which are provided first by the primitive wave and then by the well-characterized erythro-myeloid progenitor of the second wave, which does not seem to possess lymphoid potential and already arises at E8.25 (reviewed in Frame et al., 2013). Whether these two important early progenitors, the erythro-myeloid progenitor and the immune-restricted progenitor, share a common origin and might even have differentiated from the same precursors remains to be explored.

The immune-restricted progenitor identified in this study may also prove relevant to the study of pediatric leukemias, some of which are known to initiate in utero. While most patients present with acute lymphoblastic leukemia, some are diagnosed with poor prognosis biphenotypic leukemia, suggesting that the cell of origin possessed both lymphoid and myeloid potentials. Since the myeloid potential of the immune-restricted progenitor identified by Böiers et al. was shown to decrease over the course of development, it may also provide crucial clues as to the timing of preleukemic translocations. For this cell to become a useful therapeutic target, however, would require further studies into its in vivo function. Nevertheless, Böiers et al.'s identification of an LMPP-like cell present in the mammalian embryo as early as E9.5, prior to the emergence of HSCs, represents an important study as it sheds light on the development of both the myeloid innate and lymphoid adaptive immune system and describes the myeloid-lymphoid versus MkE restriction as one of the earliest hematopoietic lineage decisions. It also highlights the role of the yolk sac in setting up the first hematopoietic/immune system during development.

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Trivalent Chromatin Marks the Way iN

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Recently in *Cell*, Wapinski et al. (2013) investigated the epigenetic mechanisms underlying the direct conversion of fibroblasts to induced neurons (iNs). They found that Ascl1 acts as a pioneer factor at neurogenic loci marked by a closed "trivalent" chromatin state in cells permissive to direct conversion, but not in restrictive cell types.

Lineage-specific transcription factors define cell fate during development. Intuitively, ectopic overexpression of these transcription factors can redirect cell fate. One of the most dramatic examples of engineered cell fate change is the derivation of induced pluripotent stem cells (iPSCs) by four transcription factors (classically Oct4, Sox2, Klf4, and c-Myc) that reprogram fully differentiated cells to a pluripotent state (Takahashi and Yamanaka, 2006). This successful cellular conversion to pluripotency has also encouraged efforts to directly reprogram one cell type to another. Although examples of direct reprogramming, or transdifferentiation, already existed prior to the advent of iPSC technology (Graf, 2011), they have primarily involved switching between related cells in a lineage within the same germ layer, such as the

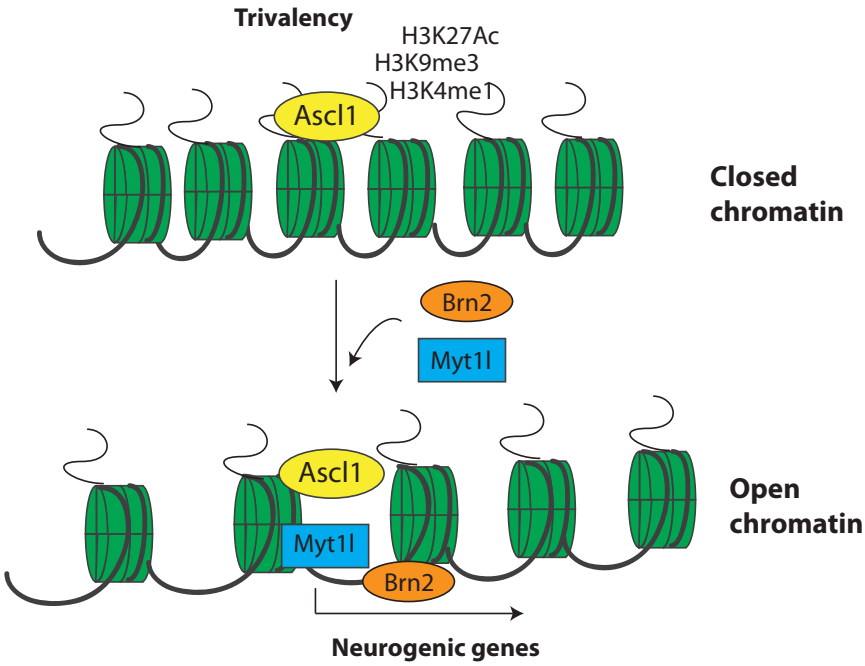

Figure 1. Pioneer Transcription Factor Ascl1 Is Recruited to Trivalent Chromatin with H3K9me3, H3K27ac, and H3K4me1 Histone Marks

Brn2 and other transcription factors are recruited to further promote transcriptional activation for neuronal conversion.

conversion of murine embryonic fibroblasts to myoblasts through overexpression of MyoD (Davis et al., 1987), or mature murine B cells to macrophages through overexpression of C/EBPs (Xie et al., 2004). In 2010, the direct reprogramming field reached an important milestone when Vierbuchen et al. (2010) found that three transcription factors (Ascl1, Brn2, and Myt1l) are sufficient to convert mesodermal murine fibroblasts to ectodermal neurons. Since this report, several groups have successfully converted somatic cells from various tissue sources into a variety of neuronal subtypes (Yang et al., 2011). In a recent issue of *Cell*, Wapinski et al. (2013) begin to address the mechanism whereby Ascl1, Brn2, and Myt1l confer neuronal identity to murine fibroblasts. They report that Ascl1 is a pioneer transcription factor occupying closed chromatin regions containing H3K4me1, H3K27ac, and H3K9me3, which subsequently recruits the other factors to activate neural pathways.

Of the three neurogenic factors, Ascl1 was already known to be essential for inducing neuronal fate because its overexpression alone can induce small neuronal features in fibroblasts (Vierbuchen et al., 2010). Wapinski et al. (2013) demonstrate that Ascl1 acts as a transcription activator that is responsible for most of the global transcriptional and genome-wide occupancy changes during iN conversion. ChIP-Seq analyses of Ascl1 binding revealed that Ascl1 occupies its targets in MEFs regardless of whether Ascl1 is expressed alone or with all three factors (Figure 1). In addition, Ascl1 seems to occupy its physiological targets in MEFs, since Ascl1 binding patterns are similar in MEFs and neural progenitor cells (NPCs). Strikingly, Brn2 is misdirected to its binding targets in the absence of Ascl1, but properly recruited in its presence, providing further support for the primary role of Ascl1 in targeting loci for activation during the induced neuron (iN) fate switch.

To determine how Ascl1 can independently target neurogenic loci during reprogramming, Wapinski et al. (2013) employed formaldehyde-assisted isolation of regulatory elements followed by massive parallel sequencing (FAIRE-seq) technology that maps genome-wide nucleosome location. Unexpectedly, the authors found that Ascl1 binds more commonly to closed chromatin, while Brn2 and Myt1l occupy regions with active histone marks. These data suggest that Ascl1 acts as a pioneer factor (Zaret and Carroll, 2011) that primes fibroblast chromatin for recruitment of other transcription factors in addition to activating iN related genes.

The most exciting finding of this study involves the revelation of a trivalent chromatin state in genomic regions of MEFs that are normally occupied by Ascl1 in NPCs. While investigating whether any epigenetic marks were responsible for initially guiding Ascl1 to its intended targets, Wapinski et al. noticed an increased co-occurrence of H3K4me1, H3K27ac, and H3K9me3 histone marks in Ascl1 sites. These trivalent sites are only present in cells permissive to iN reprogramming, such as MEFs, human dermal fibroblasts, and human skeletal muscle myoblasts, but they are not present in restrictive cells such as human keratinocytes and human osteoblasts. Additionally, some Ascl1 targets not bound in MEFs but occupied in NPCs show less prevalence of the trivalent state. In support of the functional role of the trivalent mark, the authors showed that erasure of the H3K9me3 mark by histone demethylase JmjD2 reduced reprogramming efficiency of MEFs to iNs, further strengthening the link between Ascl1 accessibility in the presence of trivalency and iN reprogramming success.

Finally, in order to further explore the functional role of Ascl1 during fibroblast to neuron transdifferentiation, Wapinski et al. tested the Ascl1 downstream target Zfp238 for its ability to functionally replace Ascl1 during iN conversion. Unlike Ascl1, Zfp238 was unable to initiate reprogramming alone, but required at least the addition of Myt1l. Even though the interaction between Ascl1 and Zfp238 was not fully examined in detail, taken as a whole, these data support a central role of the Ascl1 pathway in the neuronal fate switch.

There remain, however, many interesting questions to be addressed in future studies. Ascl1 is sufficient to initiate the reprogramming process. However, when Ascl1 is expressed alone, only occasional Tuj1+ neuronal cells emerge. This observation points to the very strong supportive roles for Brn2 and Myt1l or Zic1, most

likely in the later stages of iN reprogramming. In this study, Myt1l marked a minimal number of binding sites despite much optimization effort in ChIP, while its addition to the reprogramming cocktail significantly increased reprogramming efficiency and formation of mature neurons. The low iN conversion rate (~2%–8% depending on cell type) may obscure some interactions among Ascl1 and the supportive transcription factors. Furthermore, it will be interesting to determine how global transcription and epigenetic changes occur in instances when Ascl1 is excluded from the reprogramming cocktail.

Another stimulating question from this study is the existence of trivalent chromatin states or similar unknown chromatin states that enable accessibility to pioneer factors in other transdifferentiation contexts i.e., fibroblasts to hepatocytes and cardiomyocytes, or other known and yet undiscovered conversions (Ladewig et al., 2013). Addressing the binding particulars of how Ascl1 recognizes the trivalent state could be used to predict a more widespread modus operandi of "on target" factors.

Taken as a whole, this study's accomplishments are 2-fold. First, by delving into the mechanism of iN reprogramming, this study has provided more support for the soundness of using the direct conversion method, because binding patterns of essential transcription factors resemble those found naturally, i.e., NPCs. Second, a trivalent chromatin state is uncovered that further underscores the importance of a more complex combinatorial histone code, just like the discovery of bivalent promoters in embryonic stem cells did previously (Bernstein et al., 2006).

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Responding to R-Spondin: Slit2 Potentiates Intestinal Regeneration

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Gastrointestinal toxicity of chemoradiotherapy treatment in late-stage cancer patients limits tolerable doses and effectiveness of treatments. Zhou and colleagues in *Nature* (Zhou et al., 2013) suggest that activating Robo/Slit signaling in concert with R-Spondin potentiates Wnt signaling in intestinal stem cells and drives successful intestinal regeneration and recovery.

Over the past 5 years, there has been a dramatic increase in our understanding of intestinal biology, including intestinal stem cells (ISCs), homeostasis, and transformation (reviewed in Barker et al., 2012). Culturing intestinal epithelium as organoids in vitro is now common practice for both the small intestine and colon (reviewed in Barker et al., 2012). This wealth of information suggests that there will be an opportunity to translate this knowledge into therapeutic strategies for regenerative medicine. One key area where our increased understanding should yield important insights is intestinal injury following chemoradiotherapy. Over recent years it has been shown that Wnt signaling is activated during intestinal regeneration (Ashton et al., 2010) and that R-Spondin, a Wnt agonist, can increase proliferation of the intestine and improve recovery after chemoradiotherapy (Kim et al., 2005). Despite this, R-Spondin alone does not appear sufficiently potent to work as a single agent to provide an improved clinical outcome.

To address this, Zhou and colleagues investigated pathways that may be active within ISCs that could potentiate R-Spondin action and discovered the Slit/Robo pathway as a potential collaborator (Figure 1). They first used fluorescent